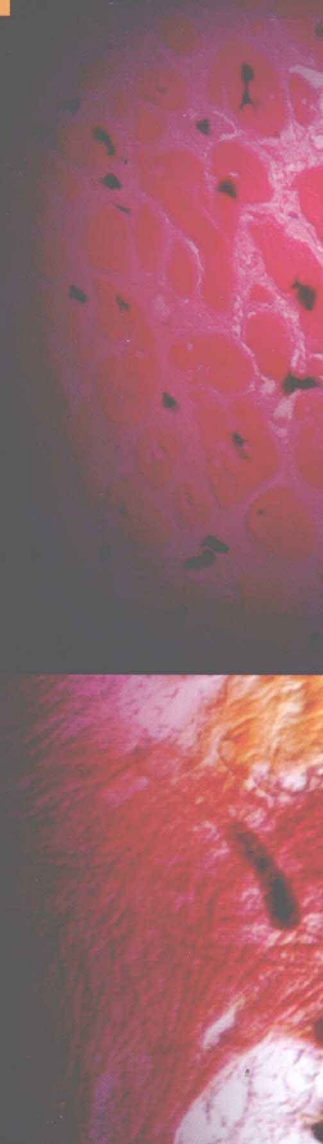
DIFFERENTIAL DIAGNOSIS IN SURGICAL PATHOLOGY

SECOND EDITION

Gattuso
Reddy
David
Spitz
Haber



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Differential Diagnosis in Surgical Pathology

Second Edition

Paolo Gattuso, MD
Professor of Pathology
Director of Anatomic Pathology
Rush Medical College of Rush University
Chicago, Illinois

Vijaya B. Reddy, MD
Professor and Associate Chair of Pathology
Rush Medical College of Rush University
Senior Attending
Rush University Medical Center
Chicago, Illinois

Odile David, MD
Director of Cytopathology
Department of Pathology
University of Illinois at Chicago
Chicago, Illinois

Daniel J. Spitz, MD
Chief Medical Examiner
Macomb County, Michigan
Clinical Assistant Professor of Pathology
Wayne State University School of Medicine
Detroit, Michigan

Meryl H. Haber, MD
Borland Professor and Chairman of Pathology, Emeritus
Rush Medical College of Rush University
Chicago, Illinois

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1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

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To my wife Nancy and my children Vincent and Francesca.

Paolo Gattuso

To my son Vikram, for the nineteen years of life, love, and memories.

Vijaya B. Reddy

For my family, to whom I owe my appreciation of life and learning.

Odile David

To my parents for setting me on the right track, and to my wife Jodi for her continuous support and encouragement.

Daniel J. Spitz

This book is dedicated to all of my former students, residents, fellows, and physician associates who I have always learned more from than I have been able to teach.

Meryl H. Haber

List of Contributors

Sylvia L. Asa, MD, PhD
Professor of Laboratory Medicine and Pathobiology
University of Toronto Faculty of Medicine
Pathologist-in-Chief
Medical Director, Laboratory Medicine Program
Senior Scientist, Ontario Cancer Institute
Toronto, Ontario
Canada
Adrenal Gland

Pincas Bitterman, MD
Professor of Pathology and Obstetrics and Gynecology
Rush Medical College of Rush University
Chicago, Illinois
Female Reproductive System

Jean E. Blair, MD
Assistant Professor of Pathology
Northeastern Ohio Universities College of Medicine
Rootstown, Ohio
Staff Pathologist
AmeriPath Youngstown
Youngstown, Ohio
Gastrointestinal System

Elizabeth J. Cochran, MD
Associate Professor of Pathology and Neurological Sciences
Rush Medical College of Rush University
Attending Physician
Rush University Medical Center
Chicago, Illinois
Central Nervous System

Kumarasen Cooper, MBChB, FRCPath, DPhil
Professor of Pathology
University of Vermont College of Medicine
Director of Anatomic Pathology and Vice-Chair of Pathology
Fletcher Allen Health Care
Burlington, Vermont
Special Diagnostic Techniques in Surgical Pathology

Byron E. Crawford, MD
Professor of Pathology
Director of Anatomic Pathology
Assistant Dean of Academic Affairs
Tulane University School of Medicine
New Orleans, Louisiana
Bones and Joints

Magdalena Czader, MD, PhD
Assistant Professor
Director, Division of Hematopathology
Director, Clinical Flow Cytometry Laboratory
Indiana University School of Medicine
Indianapolis, Indiana
Spleen

Michael J. Davis, MD
Instructor, Department of Ophthalmology
Rush Medical College of Rush University
Chicago, Illinois
Eye and Orbit

Adel K. El-Naggar, MD, PhD
Professor of Pathology and Head and Neck Surgery
Program Director, Head and Neck Pathology Fellowship
University of Texas M.D. Anderson Cancer Center
Houston, Texas
Head and Neck

Mark F. Evans, PhD
Research Assistant Professor, Department of Pathology
University of Vermont College of Medicine
Burlington, Vermont
Special Diagnostic Techniques in Surgical Pathology

Sandra E. Fischer, MD
Assistant Professor
University of Toronto Faculty of Medicine
Staff Pathologist
University Health Network
Toronto, Ontario
Canada
Adrenal Gland

Richard J. Grostern, MD
Assistant Professor of Ophthalmology and Pathology
Rush Medical College of Rush University
Chicago, Illinois
Eye and Orbit

Mehmet Guler, MD, PhD
GI/Liver Fellow, Department of Pathology
Johns Hopkins Medical Institutions
Baltimore, Maryland
Pancreas

Ralph H. Hruban, MD
Professor of Pathology and Oncology
Johns Hopkins University School of Medicine
Attending Pathologist
The Johns Hopkins Hospital
Baltimore, Maryland
Pancreas

Aliya N. Husain, MD
Professor of Pathology
University of Chicago Pritzker School of Medicine
Chicago, Illinois
Lung and Pleura

Alexandra N. Kalof, MD
Assistant Professor of Pathology
University of Vermont College of Medicine
Attending Physician, Department of Pathology
Fletcher Allen Health Care
Burlington, Vermont
Special Diagnostic Techniques in Surgical Pathology

Robin D. LeGallo, MD
Assistant Professor of Pathology
University of Virginia Medical Center
Charlottesville, Virginia
Soft Tissue

Jerome M. Loew, MD
Associate Professor of Pathology
Rush Medical College of Rush University
Assistant Attending
Rush University Medical Center
Chicago, Illinois
Lymph Nodes

Alexander Craig MacKinnon, Jr., MD, PhD
Fellow, Molecular Genetic Pathology and Cardiovascular Pathology
University of Chicago Medical Center
Chicago, Illinois
Lung and Pleura

William R. Macon, MD
Professor of Laboratory Medicine and Pathology
Mayo College of Medicine
Consultant in Pathology, Department of Laboratory Medicine and Pathology
Mayo Clinic Foundation
Rochester, Minnesota
Lymph Nodes

Cristina Magi-Galluzzi, MD, PhD
Assistant Professor
Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Director of Genitourinary Pathology
Cleveland Clinic Foundation
Cleveland, Ohio
Ureter, Urinary Bladder, and Kidney

Maria J. Merino, MD
Chief, Translational Surgical Pathology
National Institutes of Health
National Cancer Institute
Bethesda, Maryland
Breast

Cesar A. Moran, MD
Professor of Pathology and Deputy Chairman of Anatomic Pathology
University of Texas M.D. Anderson Cancer Center
Houston, Texas
Thymus and Mediastinum

Attilio Orazi, MD
Professor of Pathology and Laboratory Medicine
Weill Cornell Medical College of Cornell University
Attending Pathologist
Director, Division of Hematopathology
New York-Presbyterian Hospital
New York, New York
Spleen

Robert E. Petras, MD
Associate Clinical Professor of Pathology
Northeastern Ohio Universities Colleges of Medicine
Rootstown, Ohio
AmeriPath Gastrointestinal Institute
Oakwood Village, Ohio
Gastrointestinal System

Michael R. Pins, MD
Professor of Clinical Pathology
University of Illinois at Chicago College of Medicine
Medical Director, Anatomic Pathology
Advocate Lutheran General Hospital
Park Ridge, Illinois
Male Genitourinary System

Jose A. Plaza, MD
Assistant Professor of Pathology
Medical College of Wisconsin
Milwaukee, Wisconsin
Thymus and Mediastinum

Martha M. Quezado, MD
Staff Pathologist
Laboratory of Pathology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland
Breast

Vijaya B. Reddy, MD
Professor and Associate Chair of Pathology
Rush Medical College of Rush University
Senior Attending
Rush University Medical Center
Chicago, Illinois
Skin and Adnexal Structures

E Rene Rodriguez, MD
Adjunct and Professor
Johns Hopkins University School of Medicine
Baltimore, Maryland
Staff, Department of Anatomic Pathology
Cleveland Clinic Foundation
Cleveland, Ohio
Heart, Pericardium, and Blood Vessels

Saul Suster, MD
Chairman and Professor, Department of Pathology and Laboratory Medicine
Medical College of Wisconsin
Milwaukee, Wisconsin
Thymus and Mediastinum

Paul E. Swanson, MD
Professor of Pathology
University of Washington School of Medicine
Director of Anatomic Pathology
Director of Immunocytochemistry
University of Washington Medical Center
Seattle, Washington
Hepatobiliary System

Carmela D. Tan, MD
Staff, Section of Cardiovascular Pathology
Department of Anatomic Pathology
Cleveland Clinic Foundation
Cleveland, Ohio
Heart, Pericardium, and Blood Vessels

Mark R. Wick, MD
Professor of Pathology
University of Virginia School of Medicine
Associate Director of Virginia Health System
Charlottesville, Virginia
Soft Tissue

Michelle D. Williams, MD
Assistant Professor of Pathology
University of Texas M.D. Anderson Cancer Center
Houston, Texas
Head and Neck

Matthew M. Yeh, MD, PhD
Associate Professor of Pathology
University of Washington School of Medicine
Staff Pathologist
Gastrointestinal and Hepatic Pathology
University of Washington Medical Center
Seattle, Washington
Hepatobiliary System

Ming Zhou, MD, PhD
Assistant Professor
Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Staff Pathologist
Cleveland Clinic Foundation
Cleveland, Ohio
Ureter, Urinary Bladder, and Kidney

Preface

It has been nearly seven years since the publication of the first edition of this textbook. Who knew then that *Differential Diagnosis in Surgical Pathology* would become a textbook widely used by residents and fellows in pathology departments in the United States, as well as around the world, and by innumerable practicing pathologists. As a result of this widespread acceptance the authors began the process of updating and revising the text and illustrations while making certain to continue the successful features of the first edition, especially the organization by systems; the use of an outline format for text; and integration of the photomicrographic images with corresponding subject matter. This process took over three years.

Writing a new textbook is an immense undertaking; writing a second edition is, perhaps, an even greater undertaking. In this cost-containment environment an important consideration was to keep the book's price affordable for its audience, primarily by not increasing the number of pages (over 1000) while updating the content. This was achieved with cautious and careful editing. It was also of primary importance to fill obvious gaps and revise subject matter where needed with contributions from additional acknowledged expert pathologists. Another significant change from the first edition is the inclusion of almost all color images in this edition.

The editors, aware of the success of the book, became even more aware of some of its deficiencies or inadequacies. Every attempt to rectify any shortcomings has been made in this edition. The use of algorithms, a prominent feature of the first edition, has been dropped. Instead, each chapter outline follows a logical algorithmic approach to arriving at a correct diagnosis. The reason for this change relates to the uneven quality of several of the original algorithms and the fact that not all were useful in reaching an accurate diagnosis, mostly because of the complexity of the diagnostic problem.

The first edition's concept of brevity of each topic, not encyclopedic coverage, and outline text format accompanied by integrated illustrative examples of the pathology and limited references is maintained. It is hoped that this textbook will find its way into the hands of residents and practicing pathologists because of its concise format, excellent representative illustrations, and immediate usefulness.

Meryl H. Haber

Acknowledgments

The second edition of this textbook has greatly benefited from the expertise and experience of its many contributors throughout the United States of America and Canada. We thank them all wholeheartedly for placing their confidence in this book and sharing their knowledge so freely.

The editors gratefully acknowledge the work of authors who have contributed to this book in its previous edition.

We thank Irma Parker, Mira Davis, and Rachel Martin for their secretarial assistance. We are thankful to our publisher, Elsevier, and William R. Schmitt, Executive Editor, for his support and encouragement in the production of a second edition. A special thanks to Katie DeFrancesco, Development Editor at Elsevier, for her patience and competence in keeping the book on course over the last three years, and to Amy Norwitz, Senior Project Manager at Elsevier, who oversaw with great skill and tenacity the excruciating details that go into the final stages of the publication process.

Vijaya B. Reddy, MD

Contents

Alexandra N. Kalof, Mark F. Evans, and Kumarasen Cooper

1 Special Diagnostic Techniques in Surgical Pathology

Light Microscopy

Tissue Processing Overview

- Fixation
  - — Preserves tissues in situ as close to the lifelike state as possible
  - — Ideally, fixation will be carried out as soon as possible after removal of the tissues, and the fixative will kill the tissue quickly, thus preventing autolysis
- Dehydration
  - — Fixed tissue is too fragile to be sectioned and must be embedded first in a nonaqueous supporting medium (e.g., paraffin)
  - — The tissue must first be dehydrated through a series of ethanol solutions
- Clearing
  - — Ethanol is not miscible with paraffin, so nonpolar solvents (e.g., xylene, toluene) are used as clearing agents; this also makes the tissue more translucent
- Embedding
  - — Paraffin is the usual embedding medium; however, tissues are sometimes embedded in a plastic resin, allowing for thinner sections (required for electron microscopy [EM])
  - — This embedding process is important because the tissues must be aligned, or oriented, properly in the block of paraffin
- Sectioning
  - — Embedded in paraffin, which is similar in density to tissue, tissue can be sectioned at anywhere from 3 to 10 μm (routine sections are usually cut at 6 to 8 μm)
- Staining
  - — Allows for differentiation of the nuclear and cytoplasmic components of cells as well as the intercellular structure of the tissue
- Cover-slipping
  - — The stained section on the slide is covered with a thin piece of plastic or glass to protect the tissue from being scratched, to provide better optical quality for viewing under the microscope, and to preserve the tissue section for years

Fixation

- There are five major groups of fixatives, classified according to mechanism of action
  - — Aldehydes
    - ◆ Formalin
      - ◇ Aqueous solution of formaldehyde gas that penetrates tissue well but relatively slowly; the standard solution is 10% neutral buffered formalin
      - ◇ A buffer prevents acidity that would promote autolysis and cause precipitation of formol-heme pigment in the tissues
      - ◇ Tissue is fixed by cross-linkages formed in the proteins, particularly between lysine residues
      - ◇ This cross-linkage *does not harm the structure of proteins greatly, preserving antigenicity,* and is therefore good for immunoperoxidase techniques
    - ◆ Glutaraldehyde
      - ◇ The standard solution is a 2% buffered glutaraldehyde and must be cold, buffered, and not more than 3 months old
      - ◇ *Fixes tissue quickly and therefore is ideal for EM*
      - ◇ Causes deformation of α-helix structure in proteins and therefore *is not good for immunoperoxidase staining*
      - ◇ Penetrates poorly but gives best overall cytoplasmic and nuclear detail
      - ◇ Tissue must be as fresh as possible and preferably sectioned within the glutaraldehyde at a thickness of no more than 1 mm to enhance fixation
  - — Mercurials
    - ◆ B-5 and Zenker
      - ◇ Contain mercuric chloride and must be disposed of carefully
      - ◇ Penetrate poorly and cause tissue hardness but are fast and give excellent nuclear detail
      - ◇ Best application is for *fixation of hematopoietic and reticuloendothelial tissues*
  - — Alcohols
    - ◆ Methyl alcohol (methanol) and ethyl alcohol (ethanol)
      - ◇ Protein denaturants
      - ◇ Not used routinely for tissue because they dehydrate, resulting in tissues' becoming brittle and hard
      - ◇ *Good for cytologic smears because they act quickly and give good nuclear detail*
  - — Oxidizing agents
    - ◆ Permanganate fixatives (potassium permanganate), dichromate fixatives (potassium dichromate), and osmium tetroxide cross-link proteins
    - ◆ Cause extensive denaturation
    - ◆ Some of these have specialized applications but are used infrequently
  - — Picrates
    - ◆ Bouin solution has an unknown mechanism of action
    - ◆ It does almost as well as mercurials with nuclear detail but does not cause as much hardness

- Picric acid is an explosion hazard in dry form
- Recommended for fixation of tissues from testis, gastrointestinal tract, and endocrine organs

- Factors affecting fixation
  - — Buffering
    - Fixation is optimal at a neutral pH, in the range of 6 to 8
    - Hypoxia of tissues lowers the pH, so there must be buffering capacity in the fixative to prevent excessive acidity; acidity causes formation of formalin-heme pigment that appears as black, polarizable deposits in tissue
    - Common buffers include phosphate, bicarbonate, cacodylate, and veronal
  - — Penetration
    - Fixative solutions penetrate at different rates, depending on the diffusibility of each individual fixative
    - In order of decreasing speed of penetration: formaldehyde, acetic acid, mercuric chloride, methyl alcohol osmium tetroxide, and picric acid
    - Because fixation begins at the periphery, thick sections sometimes remain unfixed in the center, compromising both histology and antigenicity of the cells (important for immunohistochemistry [IHC])
    - It is important to section the tissues thinly (2 to 3 mm)
  - — Volume
    - Should be at least a 10:1 ratio of fixative to tissue
  - — Temperature
    - Increasing the temperature, as with all chemical reactions, increases the speed of fixation
    - Hot formalin fixes tissues faster, and this is often the first step on an automated tissue processor
  - — Concentration
    - Formalin is best at 10%; glutaraldehyde is generally made up at 0.25% to 4%
  - — Time interval
    - Formalin should have 6 to 8 hours to act before the remainder of the processing is begun
- Decalcification
  - — Tissue calcium deposits are extremely firm and do not section properly with paraffin embedding because of the difference in densities between calcium and paraffin
  - — Strong mineral acids such as nitric and hydrochloric acids are used with dense cortical bone because they remove large quantities of calcium at a rapid rate
  - — These strong acids also damage cellular morphology and thus are not recommended for delicate tissues such as bone marrow
    - Organic acids such as acetic and formic acid are better suited to bone marrow because they are not as harsh; however, they act more slowly on dense cortical bone
    - Formic acid in a 10% concentration is the best all-around decalcifier

Pearls

- Prolonged fixation can affect immunohistochemical results owing to alcohol precipitation of antigen at the cell surface; to optimize antigenicity of the tissue for IHC, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines recommend fixation of tissue destined for IHC in neutral buffered formalin for a minimum of 6 hours and a maximum of 48 hours (see Wolff et al, 2007)
- Urate crystals are water soluble and require a nonaqueous fixative such as absolute alcohol
- If tissue is needed for *immunofluorescence* (e.g., kidney or skin biopsies) or *enzyme profiles* (e.g., muscle biopsies), the specimen must be frozen without fixative; enzymes are rapidly inactivated by even brief exposure to fixation
- For rapid intraoperative analysis of tissue specimens, tissue can be frozen, and frozen sections can be cut with a special freezing microtome ("cryostat"); the pieces of tissue to be studied are snap-frozen in a cold liquid or cold environment (−20° to −70°C); freezing makes the tissue solid enough to section with a microtome

Histologic Stains

- The staining process makes use of a variety of dyes that have been chosen for their ability to stain various cellular components of tissue
- Hematoxylin and eosin (H&E) stain
  - — The most common histologic stain used for routine surgical pathology
  - — Hematoxylin, because it is a basic dye, has an affinity for the nucleic acids of the cell nucleus
  - — Hematoxylin does not directly stain tissues but needs a "mordant" or link to the tissues; this is provided by a metal cation such as iron, aluminum, or tungsten
  - — The hematoxylin-metal complex acts as a basic dye, and any component that is stained is considered to be *basophilic* (i.e., contains the acid groups that bind the positively charged basic dye), appearing blue in tissue section
  - — The variety of hematoxylin stains available for use is based partially on choice of metal ion used, which can vary the intensity or hue
  - — Conversely, eosin is an acid aniline dye with an affinity for cytoplasmic components of the cell

— Eosin stains the more basic proteins within cells (cytoplasm) and in extracellular spaces (collagen) pink to red *(acidophilic)*

Connective Tissue

- Elastin stain
  - — Elastin van Gieson (EVG) stain highlights elastic fibers in connective tissue
  - — EVG stain is useful in demonstrating pathologic changes in elastic fibers, such as reduplication, breaks or splitting that may result from episodes of vasculitis, or connective tissue disorders such as Marfan syndrome
  - — Elastic fibers are blue to black; collagen appears red; and the remaining connective tissue is yellow
- Masson trichrome stain
  - — Helpful in differentiating between collagen fibers (blue staining) and smooth muscle (bright red staining)
- Reticulin stain
  - — A silver impregnation technique stains reticulin fibers in tissue section black
  - — Particularly helpful in assessing for alteration in the normal reticular fiber pattern, such as can be seen in some liver diseases or marrow fibrosis
- Jones silver stain
  - — A silver impregnation procedure that highlights basement membrane material; used mainly in kidney biopsies

Fats and Lipids

- Oil red O stain
  - — Demonstrates neutral lipids in frozen tissue

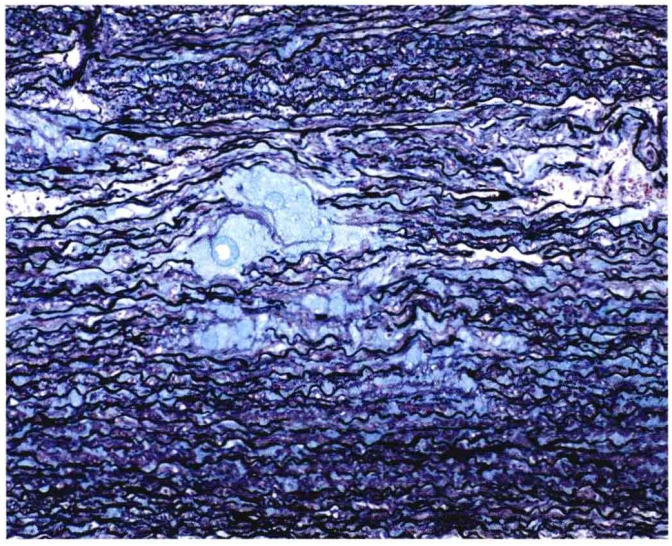

Figure 1-1. Elastin/Alcian blue stain. Aortic cystic medial degeneration in Marfan syndrome. Elastin stain highlights fragmentation of elastic fibers (*brown-black*) and pooling of mucopolysaccharides (*blue*) within the media.

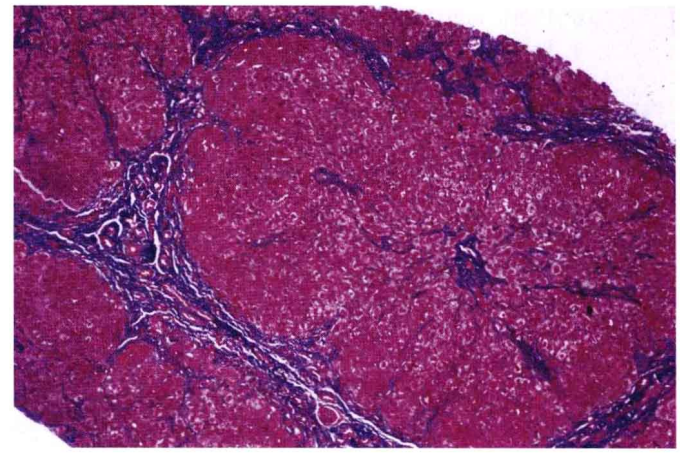

Figure 1-2. Masson trichrome stain. Cirrhosis of the liver characterized by bridging fibrosis (*blue*) and regenerative nodule formation (*red*).

- Sudan black stain
  - — Demonstrates neutral lipids in tissue sections
  - — Mainly used in hematologic preparations such as peripheral blood or bone marrow aspirations for demonstration of primary granules of myeloid lineage

Carbohydrates and Mucoproteins

- Congo red stain
  - — Amyloid is a fibrillar protein with a β-pleated sheath structure
  - — Amyloid deposits in tissue exhibit a deep red or salmon color, whereas elastic tissue remains pale pink
  - — When viewed under polarized light, amyloid deposits exhibit apple-green birefringence
  - — The amyloid fibril–Congo red complex demonstrates green birefringence owing to the parallel alignment of dye molecules along the β-pleated sheath
  - — The thickness of the section is critical (8 to 10 μm)
- Mucicarmine stain
  - — Demonstrates epithelial mucin in tissue sections
  - — Also highlights mucin-rich capsule of *Cryptococcus* species
- Periodic acid–Schiff (PAS) stain
  - — Glycogen, neutral mucosubstances, basement membranes, and fungal walls exhibit a positive PAS (bright rose)
  - — *PAS with diastase digestion*: diastase and amylase act on glycogen to depolymerize it into smaller sugar units that are then washed out of the section
  - — Digestion removes glycogen but retains staining of other substances attached to sugars (i.e., mucopolysaccharides)

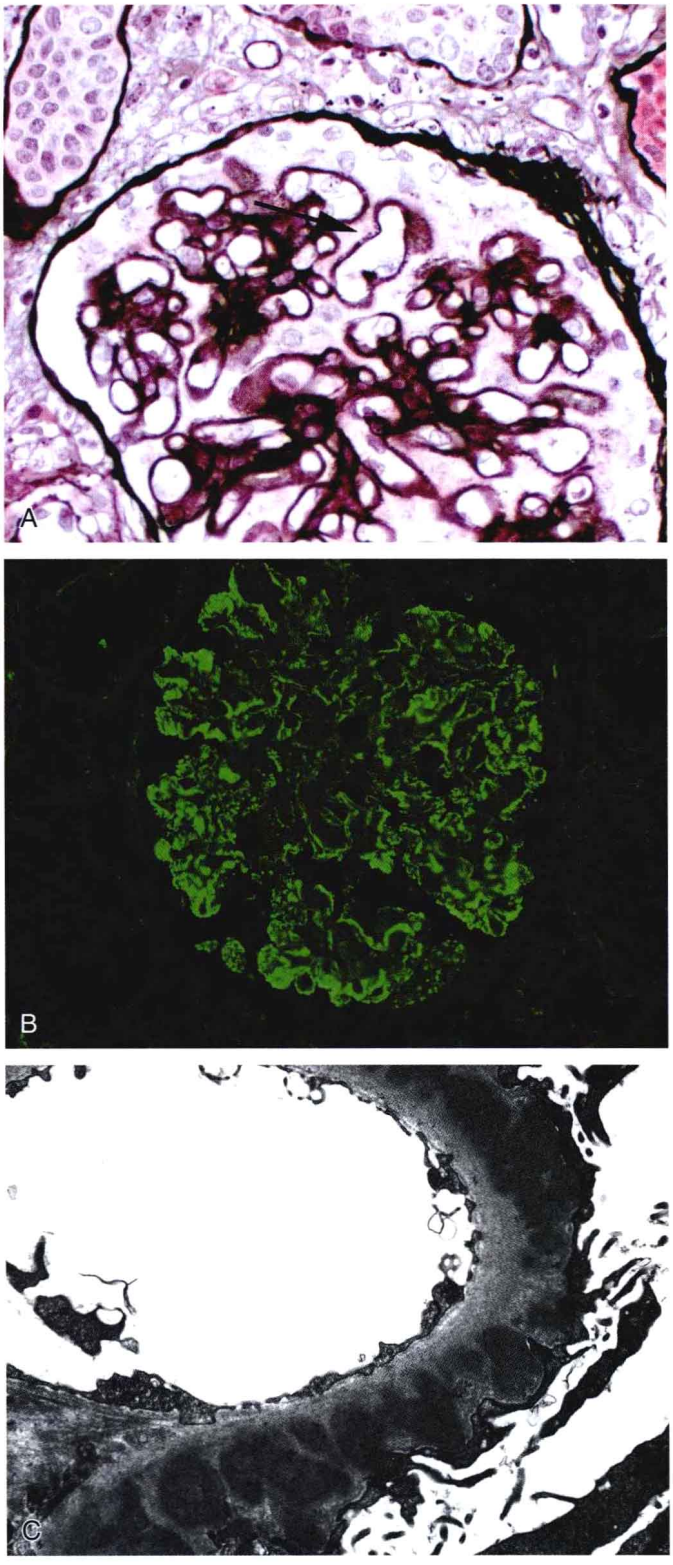

Figure 1-3. Membranous glomerulopathy. A, Jones silver stain highlighting basement membrane "spikes" (*arrow*) along glomerular capillary loops corresponding to basement membrane material surrounding intramembranous immune complexes. **B,** Direct immunofluorescence showing diffuse, granular staining of the glomerular capillary basement membranes with goat antihuman immunoglobulin G. This technique requires fresh-frozen tissue sections. **C,** Electron microscopy showing intramembranous electron-dense immune complexes within the glomerular capillary basement membranes. (Courtesy of Pamela Gibson, MD, University of Vermont/ Fletcher Allen Health Care, Department of Pathology, Burlington, VT.)

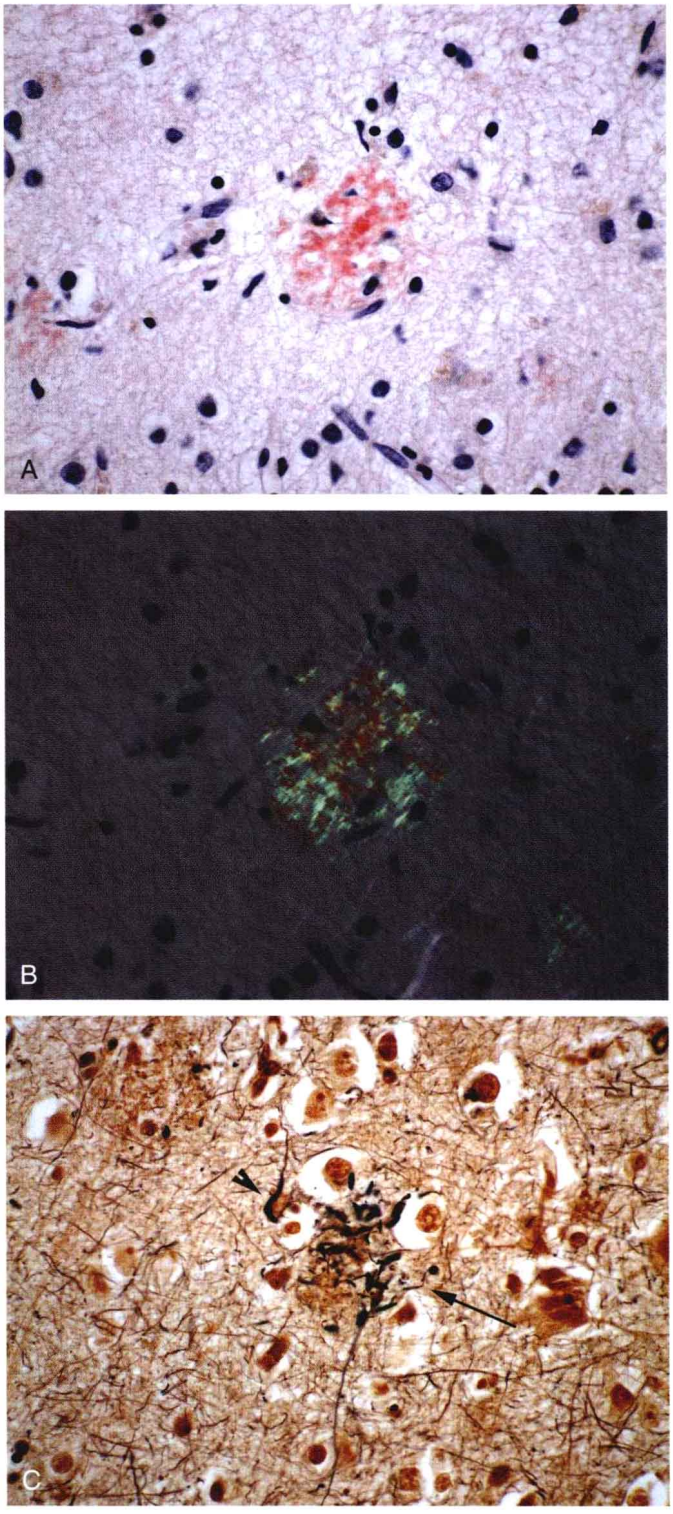

Figure 1-4. Alzheimer disease. A, Congo red–positive core of Alzheimer disease plaque. **B,** Apple-green birefringence of amyloid core under polarized light. **C,** Bielschowsky stain highlighting Alzheimer disease plaque (*arrow*) and neurofibrillary tangle within neuronal cell bodies (*arrowhead*).

- Alcian blue stain
  - — May be used to distinguish various glandular epithelia of the gastrointestinal tract and in the diagnosis of Barrett epithelium
    - pH 1.0: acid sulfated mucin positive (colonic-like)
    - pH 2.5: acid sulfated mucin (colonic-like) and acid nonsulfated mucin (small intestinal-like) positive
    - Neutral mucins (gastric-like) negative at pH 1.0 and 2.5

Pigments and Minerals

- Ferric iron (Prussian blue), bilirubin (bile stain), calcium (von Kossa), copper (rhodanine), and melanin (Fontana-Masson) are the most common pigments and minerals demonstrated in surgical pathology specimens

Nerves and Fibers

- Bielschowsky stain
  - — A silver impregnation procedure that demonstrates the presence of neurofibrillary tangles and senile plaques in Alzheimer disease
  - — Axons stain black
- Luxol fast blue stain
  - — Demonstrates myelin in tissue sections
  - — Loss of staining indicates myelin breakdown secondary to axonal degeneration
  - — Gray matter and demyelinated white matter should be almost colorless and contrast with the blue-stained myelinated white matter

Hematopoietic and Nuclear Elements

- Toluidine blue stain
  - — Demonstrates mast cells in tissue
- Giemsa, Wright, and May-Grünwald stains
  - — For cellular details, including hematopoietic (peripheral blood or bone marrow) and cytology preparations
- Leder stain (chloracetate esterase)
  - — Identification of cytoplasmic granules of granulocytes and myeloid precursors

Microorganisms: Bacteria, Fungi, Parasites

- Brown and Brenn Gram stain
  - — Demonstration of gram-negative (red) and gram-positive (blue) bacteria in tissue
- Giemsa stain
  - — Demonstration of bacteria, rickettsia, and *Toxoplasma gondii* in tissue sections
- Grocott methenamine silver (GMS) stain
  - — Demonstration of fungi or *Pneumocystis* organisms (fungi may also be demonstrated by PAS-amylase stain)
- Warthin-Starry and Steiner stains
  - — Silver impregnation technique for spirochetes (e.g., *Borrelia burgdorferi*, *Treponema pallidum*) in tissue sections
  - — *Note:* all bacteria are nonselectively blackened by silver impregnation methods such as the Warthin-Starry and Steiner stains
  - — These methods are more sensitive for small gram-negative bacteria (e.g., *Legionella* species, *Helicobacter pylori*, and *Bartonella* species) than tissue Gram stain
- Ziehl-Neelsen method for acid-fast bacteria (AFB)
  - — Detect the presence of acid-fast mycobacteria (bright red) in tissue sections (background light blue)
  - — Fite method should be used to demonstrate *Mycobacterium leprae* or *Nocardia* species, both of which are weakly acid fast

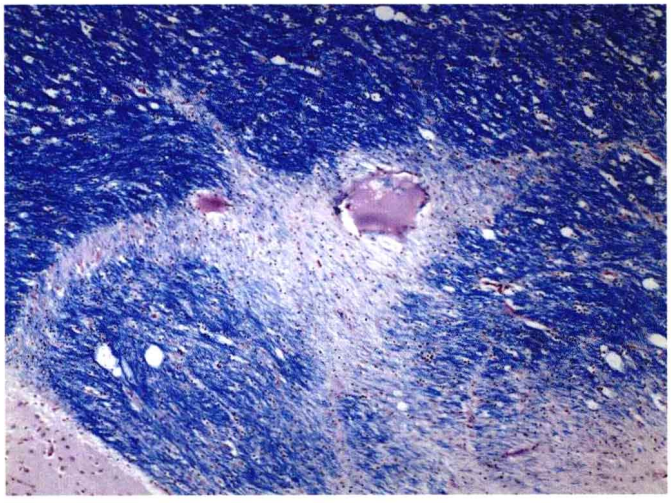

Figure 1-5. Luxol fast blue stain. Demyelination in multiple sclerosis (*colorless regions*).

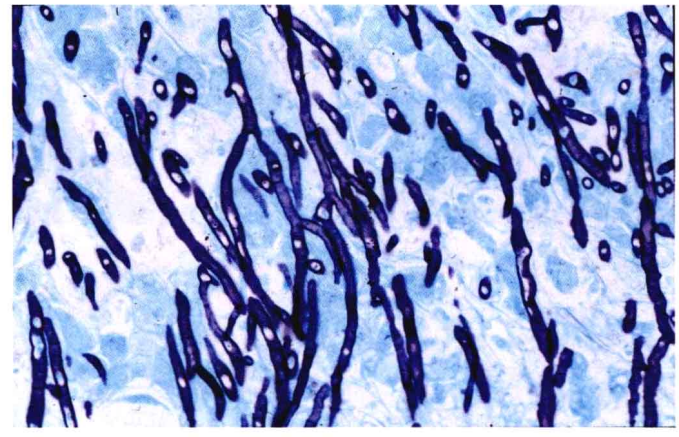

Figure 1-6. *Aspergillus* organisms in the lung stained by Grocott methenamine silver stain.

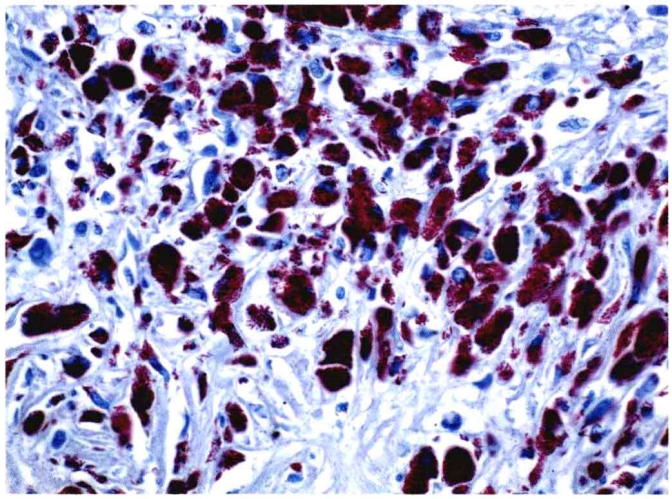

Figure 1-7. Ziehl-Neelsen stain for acid-fast bacilli. Abundant *Mycobacterium avian intracellulare* organisms (*red*) within macrophages in the lung.

Selected References

Wolff AC, Hammond ME, Schwartz JN, et al: American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. Arch Pathol Lab Med 131:18-43, 2007.

Bancroft JD, Gamble M: Theory and Practice of Histochemical Techniques, 5th ed. Philadelphia, Elsevier, 2001.

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Fluorescence Microscopy

- Tissue is exposed to short-wavelength ultraviolet (UV) light (2500 to 4000 angstroms) through a mercury or halogen lamp; the energy is absorbed by molecules that then release the energy as visible light (4000 to 8000 angstroms)
- In immunofluorescence techniques, antibodies are labeled with a fluorescent dye such as fluorescein isothiocyanate (FITC)
- Direct immunofluorescence
  - — Fluorescein-labeled antihuman globulin primary antibodies are applied to frozen, unfixed tissue sections to locate and combine with antibodies, complement, or antigens deposited in tissue
- Indirect immunofluorescence
  - — Unlabeled primary antibody is applied to the tissue section, followed by application of an FITC-labeled antibody that is directed against a portion of the unlabelled primary antibody
  - — More sensitive and more expensive
  - — Primary application in surgical pathology is detection of autoimmune diseases involving the skin and kidney (Table 1-1)

Table 1-1. Immunofluorescence Patterns and Disease Associations

Disease	Antibodies	Pattern	Histologic Manifestation
Skin			
Pemphigus vulgaris	Antidesmosomal	Intercellular chicken-wire IgG in epidermis	Suprabasal vesiculation
Bullous pemphigoid	Antiepithelial BM; anti-hemidesmosome [collagen XVII (BP180)]	Linear IgG along BM; in salt-split skin, reactivity along roof	Subepithelial vesiculation
Epidermolysis bullosa acquisita (EBA)	EBA Ag	Linear IgG along BM; in salt-split skin, reactivity along floor	Subepithelial vesiculation
Dermatitis herpetiformis	Anti-gluten	Granular IgA, especially in tips of dermal papillae	Subepithelial vesiculation
Kidney			
Anti–glomerular basement membrane (anti-GBM) disease	Anti-GBM COL4-A3 antigen	Linear GBM staining for IgG, corresponding granular staining for C3	Crescentic GN
Membranous glomerulopathy	Subepithelial deposits secondary to in situ immune complex formation (antigen unknown; associated with lupus nephritis, hepatitis B, penicillamine, gold, malignancy)	Diffuse, granular GBM staining for IgG and C3	Diffusely thickened glomerular capillary loops with lace-like splitting and "spikes" identified on Jones silver stain
IgA nephropathy	Deposited IgA polyclonal: possible increased production in response to exposure to environmental agents (e.g., viruses, bacteria, food proteins such as gluten)	IgA ± IgG, IgM, and C3 in mesangium	Focal proliferative GN; mesangial widening
Membranoproliferative glomerulonephritis	Type I: immune complex Type II: autoantibody to alternative complement pathway	Type 1: IgG + C3; C1q + C4 Type II: C3 ± IgG; no C1q or C4	Mesangial proliferation; GBM thickening; splitting

BM, basement membrane; GBM, glomerular basement membrane; GN, glomerulonephritis; Ig, immunoglobulin.